StressMarq Biosciences INC.

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StressXpress®

Glutathione Fluorescent Detection Kit

Catalog# SKT-202 (96-Well Kit)

Fluorescent detection of total GSH content

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

Catalog Number	Reagent	Quantity	Description
SKC-202A	Black 96 Well Plate	1 Each	-
SKC-202B	Glutathione Standard	100 µL	Glutathione at 250 µM in a special stabilizing solution.
SKC-202C	StressXpress® Detection Reagent	2 Plastic vials	Thiol detection substrate stored in a desiccator. Reconstitute with dry DMSO.
SKC-202D	Dry DMSO	4 mL	Dry Dimethyl sulfoxide solvent over molecular sieves. May be stored at room temperature.
SKC-202E	Assay Buffer	60 mL	A buffer containing detergents and stabilizers. Ready-to-use Assay Buffer.
SKC-202F	NADPH Concentrate	300 µL	Reduced ß-nicotinamide adenine dinucleotide 2'-phosphate (NADPH) as a stable solution.
SKC-202G	Glutathione Reductase Concentrate	300 µL	Glutathione Reductase (GR) as a stable solution.
SKC-202H	Oxidized Glutathione Control	300 µL	Oxidized Glutathione (GSSG) in a special stabilizing solution. This is an optional control solution to ensure NADPH/GR performance.

If any of the items listed above are damaged or missing, please contact our Customer Service department at (250) 294-9065. We cannot accept any returns without prior authorization.

WARNING: Not for human or animal disease diagnosis or therapeutic drug use.

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete booklet should be read and understood before attempting to use the product.

Sulfosalicylic acid is a strong acid solution and should be treated like any other laboratory acid.

Dimethyl sulfoxide is a powerful aprotic organic solvent that has been shown to enhance the rate of skin absorption of skin-permeable substances. Wear protective gloves when using the solvent especially when it contains dissolved chemicals. NOTE: DMSO can dissolve certain plastics used in troughs used for holding solutions for multichannel pipets,

StressXpress® Thiol Detection Reagent should be stored at 4°C in the desiccator. Allow desiccator to warm to room temperature prior to opening. Detection Reagent will react with strong nucleophiles. Buffers containing the preservatives sodium azide, Proclin™ and Kathon™ will react with the substrate.

Storage

All components of this kit should be stored at 4°C until the expiration date of the kit.

DMSO, when stored at 4°C, will freeze. Can be stored tightly capped at room temperature.

Materials Needed But Not Supplied

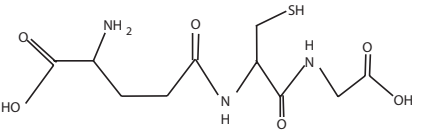
- Distilled or deionized water
- Repeater pipet with disposable tips capable of dispensing 25 µL.
- Aqueous 5-sulfo-salicylic acid dihydrate (SSA) solution at 5% weight/volume (1g of SSA per 20 mL of water) for treating samples to remove protein.
- Fluorescence 96 well plate reader capable of reading fluorescent emission at 510 nm, with excitation at 390 nm. Please contact your plate reader manufacturer for suitable filter sets. Set plate parameters for a 96-well Corning Costar 3686 plate. Plate Dimensions (in mm): Well Depth: 10.54; Plate Length: 127.8; Plate Width: 85.5; A1 Row Offset: 11.2; A1 Column Offset: 14.3.
- The sensitivity of fluorescent assays is dependant on the capabilities of the plate reader. If your plate reader has adjustable gain you can modify the signals obtained from the assay by increasing or decreasing the gain settings, by changing the aperture settings for monochromator based readers, or by changing the band pass width of the emission and/or excitation filters on some readers. Please review the plate reader manual for details.
- Signals expressed by plate readers are Relative Fluorescent Units (RFU) and the values given in the booklet were obtained on our plate readers. The RFU numbers you obtain may be different from these, but the assay results should be similar.
- Software for converting raw relative fluorescent unit (FLU) readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Please read this booklet completely prior to using the product.
FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

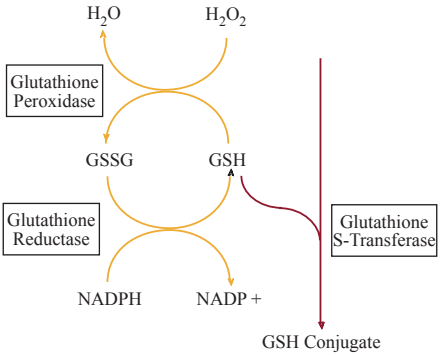
INTRODUCTION

Background

Glutathione (L-γ-glutamyl-L-cysteinylglycine; GSH) is the highest concentration non-protein thiol in mammalian cells and is present in concentrations of 0.5 – 10 mM[1]. GSH plays a key role in many biological processes, including the synthesis of proteins and DNA, the transport of amino acids, and the protection of cells against oxidation. Harmful hydrogen peroxide cellular levels are minimized by the enzyme glutathione peroxidase (GP) using GSH as a reductant[2].



The oxidized GSH dimer, GSSG, is formed from GSH and peroxide by the GP reaction (see below). An important role of GSSG in the NFγB activating signal cascade is suggested by the facts that the potent NFγB inducer, tetradecanoyl phorbol acetate, increases intracellular GSSG levels and GSSG/GSH ratios[3].



Glutathione S-transferases (GST) are an important group of enzymes that catalyze the nucleophilic addition of GSH to electrophiles. They are encoded by 5 gene families; 4 encode cytosolic GST and one encodes the microsomal form of GST. They have been implicated in a number of diseases. In asthma arachidonic acid is converted to unstable leukotriene A_4 (LTA_4). LTA_4 is either hydrated to form LTB_4 or it is conjugated to GSH by a GST, leukotriene C_4 synthase, to form leukotriene C_4. LTC_4 and its derivative LTD_4 are important molecules in bronchial asthma. Leukotriene C_4 synthase is therefore an important therapeutic target. It has also been shown that increased expression of GSTs can lead to drug resistance. Three glutathione adducts of the drug melphalan, used to treat ovarian cancer and multiple myeloma, have been isolated from reactions involving human microsomal GSTs.

Assay Overview

The Glutathione *StressXpress*® Fluorescent Detection kit is designed to quantitatively measure glutathione (GSH), and oxidized glutathione (GSSG) present in a variety of samples. The kit is unique in that both free and oxidized glutathione are detected in the same well in the microtiter plate. No separation or washing is required. Total glutathione is the sum of GSSG plus GSH. Please read the complete kit booklet before performing this assay. A GSH standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. The kit utilizes a proprietary non-fluorescent molecule, StressXpress® Detection Reagent, that will covalently bind to the free thiol group on GSH to yield a highly fluorescent product. After mixing the sample or standard with the Detection Reagent and incubating at room temperature for 15 minutes, the fluorescent product is read at 510 nm in a fluorescent plate reader with excitation at 390 nm. The concentration of the GSH in the sample is calculated, after making a suitable correction for any dilution of the sample, using software available with most fluorescence plate readers.

Free glutathione, GSH, is read first after 15 minutes, followed by addition of a reaction mixture that converts all the oxidized glutathione, GSSG, into free GSH, which then reacts with the excess Detection Reagent to yield the signal related to Total GSH content. The total concentration of GSH generated in the sample is calculated from the generated signal. We have provided a 96 well plate for measurement but this assay is adaptable for higher density plate formats. The end user should ensure that their HTS black plate is suitable for use with these reagents prior to running samples.

Sample Types

Sample Types Validated:
Whole Blood, Serum, Plasma, Erythrocytes, Urine, Cell Lysates and Tissue Samples

GSH is identical across species and we expect this kit may measure GSH from sources other than human. The end user should evaluate recoveries of GSH in samples from other species being tested.

If samples need to be stored after collection, we recommend storing them at -70°C or lower, preferably after being frozen in liquid nitrogen. This assay has been validated for human whole blood, serum, EDTA and heparin plasma, urine, and isolated erythrocytes. Most cell lysates and tissue homogenates should also be compatible. Samples containing visible particulate should be centrifuged prior to using.

All samples will be deproteinized with 5% SSA (see page 5 for preparation), please see sample specific information below for details. This treatment removes any protein thiols present in the samples and also slows oxidation of free GSH.

Sample Preparation

All samples must be treated with the SSA solution prepared on page 5. All of the SSA treated centrifuged supernatants must have their SSA concentration brought down to 1% SSA by dilution with Assay Buffer. Further dilutions of the sample, using Sample Diluent (see page 13 for preparation), may be necessary to allow the GSH concentration to be measurement in the assay. Detailed instructions follow.

All samples and standards must be in Sample Diluent before starting the assay.

Use all samples within 2 hours of dilution.

Whole Blood, Serum, EDTA or Heparin Plasma, or Urine

Thoroughly mix sample with an equal volume of cold 5% SSA. Incubate for 10 minutes at 4°C. Centrifuge at 14,000 rpm for 10 minutes at 4°C. Collect the supernatant. If the supernatent contains particulates, re-centrifuge the supernatant for 15 minutes and collect the clarified second supernatant. Samples can be stored in aliquots at ≤ -70°C or analyzed immediately. At this point the SSA concentration will be 2.5%.

The supernatant must be diluted 1:2.5 with Assay Buffer by mixing one part with 1.5 parts of Assay Buffer. The SSA concentration will be 1%. The sample will have been diluted 1:5 at this point.

All final dilutions are to be made in Sample Diluent. Treated Whole Blood must be further diluted at least 1:20 for a recommended final dilution of ≥ 1:100. For Treated Plasma and Treated Urine a final dilution of ≥ 1:5 is recommended, but further dilutions in Sample Diluent may be necessary.

Tissue Samples

Fresh tissue is washed with ice cold PBS to remove blood then blotted on filter paper before recording wet weight. NOTE: Samples that have been frozen will contain lysed cells. The PBS wash may contain substantial amounts of GSH and/or GSSG.

- For Samples Where a Protein Determination is to be Obtained: Homogenize at 10 mg/250 µL in ice cold 100mM phosphate buffer, pH 7. Centrifuge at 14,000 rpm for 10 minutes at 4°C and remove an aliquot of the supernatant for protein determination. Thoroughly mix a second aliquot of the supernatant with an equal volume of cold 5% SSA. Incubate for 10 minutes at 4°C. Centrifuge at 14,000 rpm for 10 minutes at 4°C to remove precipitated protein. Collect the supernatant. The supernatant must be diluted 1:2.5 with Assay Buffer by mixing one part with 1.5 parts of Assay Buffer. The SSA concentration will be 1%.

- For Samples Not Requiring a Protein Determination: Homogenize at 10 mg/250 µL in ice cold 5% SSA, incubate at 10 minutes at 4°C, then centrifuge at 14,000 rpm for 10 minutes at 4°C to remove precipitated protein. Collect the supernatant. The supernatant must be diluted 1:5 with Assay Buffer by mixing one part with 4 parts of Assay Buffer. The SSA concentration will be 1%.

Further sample dilutions must be determined by the end-user since it will be dependent upon the tissue type and the amount of tissue used. These dilutions must be made in the prepared Sample Diluent.

Erythrocytes, Red Blood Cells (RBC's)

Collect blood with heparin or EDTA. Centrifuge the sample, remove and discard the plasma and white cell layer. Wash the RBC's 2 times by suspending in 3 volumes of isotonic saline (0.9%), centrifuging at 600 x g for 10 minutes and discarding the saline wash.

After the 2 washes, mix 250µL RBC's with 1mL of cold 5% SSA. Incubate for 10 minutes at 4°C. Centrifuge at 14,000 rpm for 10 minutes at 4°C. Collect the supernatant. At this point the SSA concentration will be 4%. The supernatant must be diluted 1:4 with Assay Buffer by mixing one part with 3 parts of Assay Buffer. The SSA concentration will now be 1%. The sample will have been diluted 1:20 at this point. Further dilutions are made in Sample Diluent. NOTE: Human RBC's require a final dilution of 1:100-1:200 to read within the standard curve.

Cell Lysates

Washed cell pellets are resuspended at 1-10x10^6 cells/mL in cold 5% SSA (we used Jurkats at 5x10^6 cells/mL) and are lysed and deproteinized by vigorous vortexing, freeze/thaw cycling or other suitable disruption method. Incubate cells at 4°C for 10 minutes followed by centrifugation for 10 minutes at 14,000 rpm and 4°C. NOTE: Samples that have been frozen will contain lysed cells. The PBS wash may contain substantial amounts of GSH and/or GSSG.

The centrifuged supernatants must be diluted 1:5 with Assay Buffer by mixing one part with 4 parts of Assay Buffer. The SSA concentration will be 1%. The sample will have been diluted 1:5 at this point. Further sample dilutions must be done in Sample Diluent and need to be determined by the end-user since it will be dependent upon the cell type and number of cells used. The recommended final dilution is ≥ 1:20.

Use all samples within 2 hours of dilution.

Reagent Preparation

Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine GSH concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

Do not dilute the Assay Buffer.

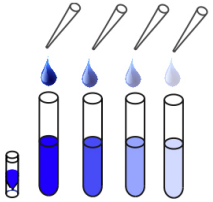
Sample Diluent

Prepare the Sample Diluent by diluting one part 5% SSA 1:5 with four parts Assay Buffer and vortex thoroughly. The pH of the Sample Diluent must be > 6. Sample Diluent can be stored at 4°C for one month.

Standard Preparation

GSH Standards are prepared by labeling eight test tubes as #1 through #8. Briefly vortex to mix and then spin the vial of standard in a microcentrifuge to ensure contents are at bottom of vial. Pipet 450 µL of Sample Diluent into tube #1 and 250 µL into tubes #2 to #8. Carefully add 50 µL of the Glutathione Standard to tube #1 and vortex completely. Take 250 µL of the GSH solution in tube #1 and add it to tube #2 and vortex completely. Repeat this for tubes #3 through #8. The concentration of GSH in tubes 1 through 8 will be 25, 12.5, 6.25, 3.125, 1.56, 0.781, 0.391 and 0.195 µM.

Use all Standards within 1 hour of preparation.



	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8
Sample Diluent Volume (µL)	450	250	250	250	250	250	250	250
Addition	Stock	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
Volume of Addition (µL)	50	250	250	250	250	250	250	250
Final Concentration (µM)	25	12.5	6.25	3.125	1.56	0.781	0.391	0.195

Control Preparation (Optional)

This optional control solution for ensuring complete conversion of GSSG to GSH can be prepared by adding 5 µL of Oxidized Glutathione Control to 245 µL of Sample Diluent.
Use within 2 hours.

StressXpress® Detection Reagent

Allow the desiccator to warm to room temperature prior to opening and remove the vial of Detection Reagent. Add the volume of DMSO provided to the vial according to the table below. Vortex thoroughly. Store any unused reconstituted Detection Reagent at 4°C in the desiccator and use within 2 weeks.

Vial Part Number	SKC-202C-1EA, Plastic vial
Volume of DMSO to add per vial	1.5 mL
For # of Wells	Up to 60

Reaction Mixture

Prepare the Reaction Mixture by vortexing the vials of Glutathione Reductase and NADPH Concentrates and then diluting one part each NADPH and Glutathione Reductase Concentrates 1:10 into eight parts Assay Buffer. Vortex thoroughly. See Table for suitable volumes. Store any unused Reaction Mixture at 4°C in an amber vial for no more than 2 days.

Reaction Mix Dilution Table

	1/2 Plate	One Plate
NADPH Concentrate	150 µL	275 µL
Glutathione Reductase Concentrate	150 µL	275 µL
Assay Buffer	1.2 mL	2.2 mL

Assay Protocol - Free and Total GSH

1. Use the plate layout sheet on page 29 to aid in proper sample and standard identification. Set plate parameters for a 96-well Corning Costar 3686 plate. Plate Dimensions (in mm): Well Depth: 10.54; Plate Length: 127.8; Plate Width: 85.5; A1 Row Offset: 11.2; A1 Column Offset: 14.3.
2. Pipet 50 µL of treated samples, standards or control into wells in the plate.
3. Pipet 50 µL of Sample Diluent into Zero wells in the plate.
4. Add 25 µL of the StressXpress® Detection Reagent to each well using a repeater pipet.
5. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
6. Incubate at room temperature for 15 minutes.
7. Read the fluorescent signal from each well in a plate reader capable of reading the fluorescent emission at 510 nm with excitation at 370-410 nm. This data will be used to determine Free GSH concentration.
8. Add 25 µL of the Reaction Mixture to each of the wells using a repeater pipet.
9. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
10. Incubate at room temperature for 15 minutes.
11. Read the fluorescent emission at 510 nm with excitation at 370-410 nm. This data will be used to determine Total GSH concentration.

Total GSH Content Only

Total GSH content can be determined directly by leaving out steps 5, 6 and 7.

ANALYSIS

Calculation of Results

Average the duplicate FLU readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean FLUs for the zero standard. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

Free glutathione (GSH) concentrations are calculated from the data obtained from step 7 on page 16 utilizing the curve fitting routine supplied with the plate reader.

Total glutathione concentrations of the samples are calculated from the data obtained from step 11 on page 16 utilizing the curve fitting routine supplied with the plate reader. Ensure that the Reaction Mixture is added to all the wells used, including the standard and control wells. The volumes must be the same in the standard, control and samples wells.

Oxidized glutathione (GSSG) concentrations are obtained by subtracting the Free GSH levels from the Total GSH concentrations and dividing by 2. See Below:

$$GSSG = \frac{(\text{Total GSH} - \text{Free GSH})}{2}$$

TOTAL GSH NOTE:

When Free GSH and Total GSH levels are almost identical, we suggest that you block the free GSH by addition of 2-Vinylpyridine (2VP) to an aliquot of the sample. 2VP is prepared by adding 27 µL of 2-vinylpyridine to 98 µL of ethanol. Use immediately and discard remaining unused solutions.

2VP is TOXIC and may cause burns. 2VP solutions should be prepared in a fume hood. Use immediately and discard remaining unused solutions by mixing with copious amounts of water.

To 250 µL of 5% SSA treated samples add 5 µL of the ethanolic solution of 2VP and allow to incubate at room temperature for 1 hour. The 2VP treated samples should then be diluted in Assay Buffer and Sample Diluent according to the dilutions recommended for each sample type on pages 9 and 10 prior to using in the assay.

Typical Data - Free GSH

Sample	Mean FLU	Net FLU	GSH Concentration (µM)
Zero	299	0	0
Standard 1	40,945	40,646	25
Standard 2	19,737	19,438	12.5
Standard 3	10,006	9,707	6.25
Standard 4	5,269	4,970	3.125
Standard 5	2,671	2,372	1.56
Standard 6	1,571	1,272	0.781
Standard 7	1,009	710	0.391
Standard 8	639	394	0.195
Sample 1	1,568	1,269	0.77
Sample 2	5,448	5,149	3.41

Always run your own standard curve for calculation of results.
Do not use this data.

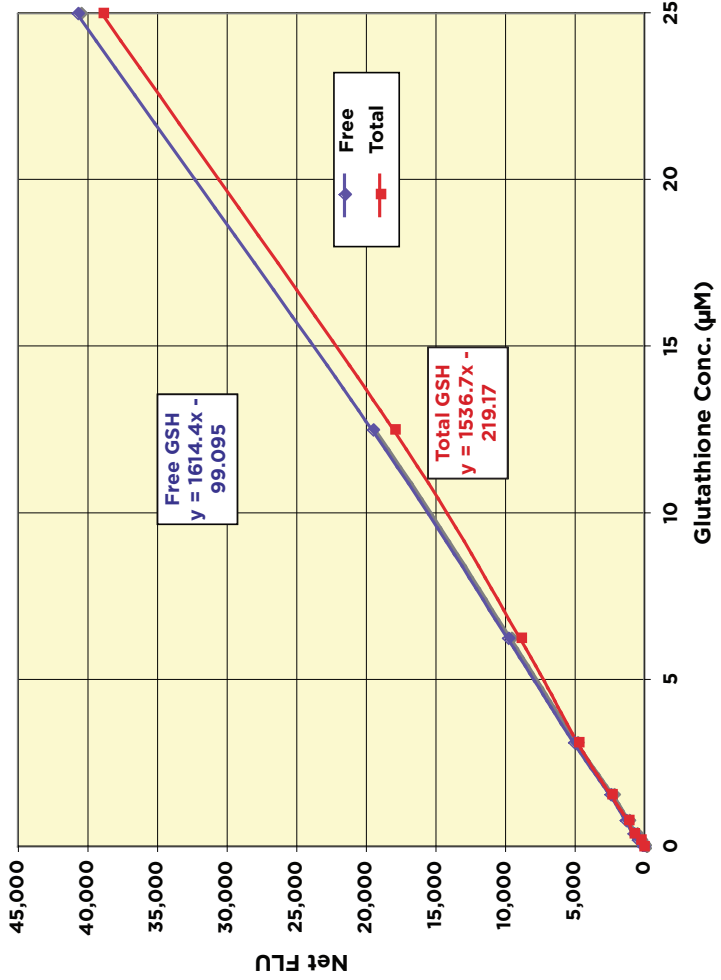
Typical Data - Total GSH

Sample	Mean FLU	Net FLU	GSH Concentration (µM)
Zero	1,127	0	0
Standard 1	39,976	38,849	25
Standard 2	19,034	17,907	12.5
Standard 3	9,958	8,831	6.25
Standard 4	5,814	4,687	3.125
Standard 5	3,429	2,302	1.56
Standard 6	2,209	1,082	0.781
Standard 7	1,816	689	0.391
Standard 8	1,339	212	0.195
Sample 1	4,553	3,426	2.53
Sample 2	10,428	9,301	6.64

Always run your own standard curve for calculation of results.
Do not use this data.

See NOTE on page 17 concerning Free and Total GSH levels in samples.

Typical Standard Curves



Validation Data

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the FLUs for twenty wells run for each of the zero and standard #8. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.
Sensitivity was determined as 45 nM in the Free GSH and 48 nM in the Total GSH assays.

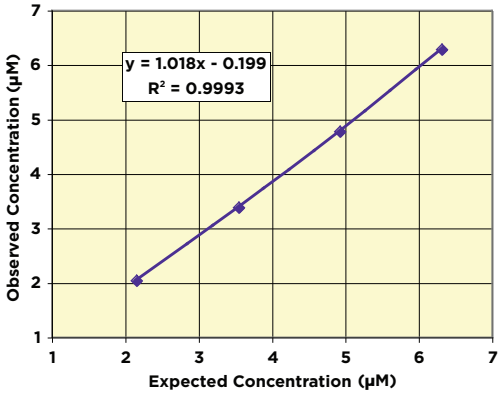
The Limit of Detection was determined in a similar manner by comparing the FLUs for twenty wells run for each of the zero and a low concentration human serum sample.
The Limit of Detection was determined as 38 nM in the Free GSH and 42 nM in the Total GSH assays.

Linearity

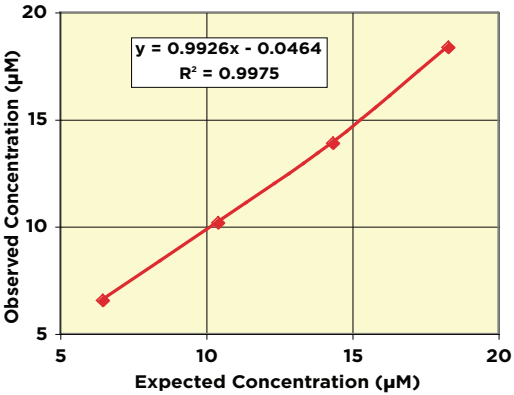
Linearity was determined by taking human RBCs at two different concentrations and mixed in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High RBC Sample	Low RBC Sample	Observed Concentration (µM)		Expected Concentration (µM)		% Recovery	
		Free	Total	Free	Total	Free	Total
100%	0%	7.71	22.23	--	--	--	--
80%	20%	6.28	18.35	6.32	18.29	99.3%	100.3%
60%	40%	4.77	13.89	4.93	14.35	96.7%	96.8%
40%	60%	3.38	10.18	3.55	10.41	95.3%	97.8%
20%	80%	2.04	6.55	2.16	6.47	94.5%	101.2%
0%	100%	0.77	2.53	--	--	--	--
				Mean Recovery		96.5%	99.0%

Free GSH Linearity



Total GSH Linearity



Intra Assay Precision

Two each of SSA treated human urine and whole blood samples were further diluted in 1% SSA Sample Diluent and run in replicates of 20 in an assay. The mean and precision of the calculated GSH concentrations were:

Sample	GSH Concentration (µM)		%CV	
	Free	Total	Free	Total
1	1.27	2.30	4.0	4.7
2	2.00	3.80	3.1	4.7
3	8.33	9.77	4.6	2.7
4	3.89	4.45	3.0	2.3

Inter Assay Precision

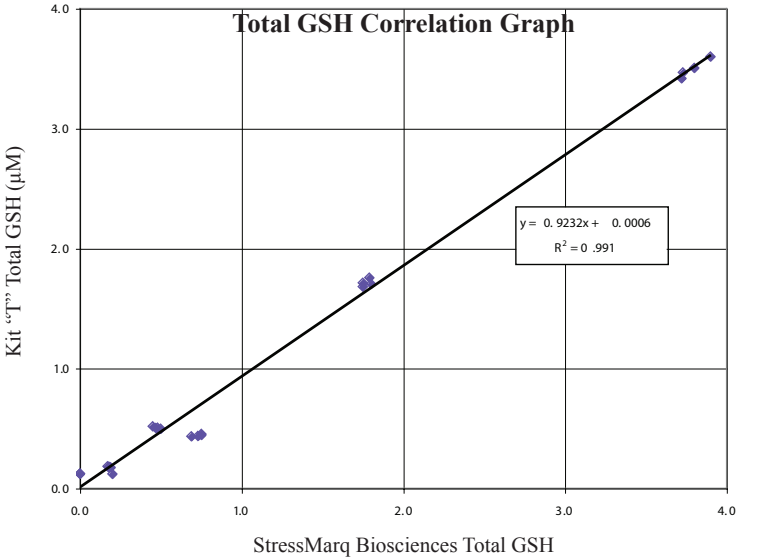
Two each of SSA treated human urine and blood samples were further diluted in 1% SSA Sample Diluent and run in duplicates in twenty assays run over multiple days by two operators. The mean and precision of the calculated GSH concentrations were:

Sample	GSH Concentration (µM)		%CV	
	Free	Total	Free	Total
1	1.30	2.40	8.6	8.3
2	1.83	3.57	14.7	10.0
3	9.38	11.67	6.0	6.0
4	4.89	5.89	7.2	8.0

Kit Correlation Data

We purchased and compared a popular colorimetric total glutathione assay kit (kit "T") that uses Ellman's reagent to detect free glutathione in the sample. Initial experiments used random human urine samples that were processed as described in each kit booklet. With kit "T", the values obtained for urine after the recommended treatment with 4 volumes of 5% metaphosphoric acid and subsequent 10 fold dilution with assay buffer put all the values well below the lowest standard. However, the urine samples run in the StressXpress® kit gave Total GSH values between 0.63 and 4.04 μM.

We also took a Jurkat cell pellet and processed the cells either through the 5% metaphosphoric acid treatment for the kit "T" Ellman's based test or as described on page 13 for the StressXpress® kit. Cell samples ranged from 25 to 0.78 x 10^6 cell/mL. Twenty-four samples were run according to manufacturers directions for both kits and the correlation of these samples is shown below.



Many of the cell lysate values for the Ellman's based kit, kit "T", read either below the lowest standard (0.25 µM) or above the highest one (2 µM). This data was calculated via extrapolation from the kinetic method required by kit "T". The lysate values for the StressXpress® kit were calculated directly from the endpoint standard curve.

References

1. Meister, A. “On the Discovery of Glutathione.” Trends Biochem. Sci. 1988 13(5): 185-188.
2. Meister, A. “The Glutathione-Ascorbic Acid Antioxidant Systems in Animals” J. Biol. Chem. 1994 269:9397-9400.
3. Dröge W, et al., “Functions of Glutathione and Glutathione Disulfide in Immunology and Immunopathology” FASEB J., 1994 8:1131-1138.

Warranty and Limitation of Remedy

StressMarq Biosciences Inc. makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. StressMarq **warrants only** to the original customer that the material will meet our specifications at the time of delivery. StressMarq will carry out its delivery obligations with due care and skill. Thus, in no event will StressMarq have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if StressMarq is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of StressMarq, its directors or its employees.

Buyer's **exclusive remedy** and StressMarq's sole liability hereunder shall be limited to a refund of the purchase price, or at StressMarq's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to StressMarq within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Refund Policy located on our website and in our catalog.

Contact Information

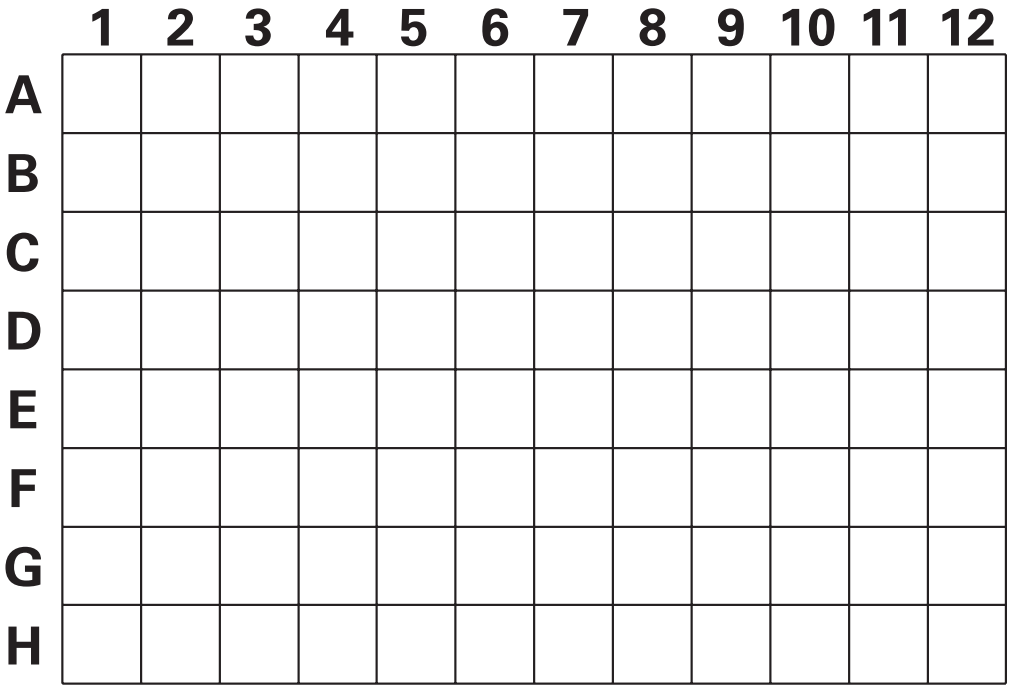
Phone: 250-294-9065

Fax: 250-294-9025

E-Mail: techsupport@stressmarq.com

Hours: M-F 9:00 AM to 5:00 PM PST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).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NOTES